REMARKS / ARGUMENTS

The Claims

Claims 61-68 are currently pending in the application and are directed to a method of treating bone loss by administering an expression vector comprising a nucleic acid encoding osteoprotegerin.

Compliance under Rules for disclosure of nucleotide and/or amino acid sequences 37 CFR 1.821-1.825

The application is alleged not to be in compliance with the requirements for disclosure of sequences because sequence identifiers are missing from Figures 1A-B, 2B-E, 9A-F, 10, 12A-B and 14A. Applicants have amended the specification to include sequence identifiers for the figures.

In order to bring the application into compliance with 37 CFR1.821-1.835, Applicants submit herewith a substitute sequence listing and computer readable form (CRF), and a statement declaring that the contents of the substitute sequence listing and CRF are the same.

Rejections under 35 U.S.C. 112

Claims 61-68 are rejected under 35 U.S.C. 112, first paragraph, as allegedly containing subject matter which the specification does not enable one skilled in the art to make and/or use commensurate in scope with the claims.

The claims are directed to a method for treating bone loss comprising administering to a mammal an expression vector comprising a nucleic acid sequence encoding osteoprotegerin (OPG). In Example 3, one such method is shown, namely the production of transgenic mice which express OPG and show increased bone density. Example 11 also describes experiments which demonstrate that OPG can be used to treat bone loss (see, for instance, Example 11D on p. 132 of the specification which describes effects of OPG on bone loss in ovariectomized rats). These teachings clearly point to a working method for treating bone loss by administering a nucleic acid sequence encoding OPG and therefore provide an enabling disclosure. The Federal Circuit has held that "[t]he enablement requirement is met if the description enables any mode of making and using the invention" *Engel Indus., Inc. v. Lockformer Co.* 20 USPQ2d 1300, 1304 (Fed. Cir. 1991). Applicants have clearly enabled at least one mode of making and using the invention and have satisfied the enablement requirement.

Applicants maintain that the Examiner has not established a *prima facie* case of nonenablement. The burden falls on the Examiner to establish reasons as to why the specification does not enable the claimed invention and the Examiner has failed to do so. *In re Marzochi* 169 USPQ 367 (CCPA, 1971).

The Examiner argues that "[c]laims 61-68 are directed to somatic cell gene therapy." While somatic cell gene therapy could be considered one embodiment of Claims 61-68, the claims are by no means restricted to such a method. The allegation by the Examiner that "a transgenic mouse is not predictive of ... gene

therapy" is not a proper argument for nonenablement. As discussed above, Applicants have enabled one embodiment of the claimed method and unsupported arguments that another embodiment of the claim, gene therapy, may not be predicted to work is not a basis for alleging lack of enablement.

It is also argued that the claimed method should be carried out in a disease model, rather than in normal animals. Applicants maintain that the teachings in the specification taken together do not require such a showing. The increase in bone density in OPG transgenic mice as shown in Example 3 together with the observed reduction in bone loss by OPG injected into ovariectomized mice as shown in Example 11D, clearly conveys to one skilled in the art that the introduction of a nucleic acid encoding OPG into an animal experiencing bone loss would result in the reduction of bone loss. The effects of OPG, whether administered by introduction of a nucleic acid expressing OPG or an OPG protein, would clearly be expected to be the same. This expectation is also supported in the specification at p. 133, line 11, where it is stated that "[t]he in vivo actions of recombinant OPG parallel the changes seen in OPG transgenic mice."

It is also argued that somatic cell gene therapy is unpredictable and that the selection of vectors and promoters is "very important". Applicants reiterate that somatic cell gene therapy is but one embodiment of the claims. Nonetheless, vectors for gene therapy were available as of the priority date of the application. For example, numerous references published prior to 1995 showed examples of successful gene therapy (see review by Yang et al. (Critical Rev. Biotech. 12, 335-356 (1992) attached hereto as Exhibit A)). As stated in the abstract of the Yang reference on p. 335:

> Genetically engineered retroviral vectors have been used successfully to infect live animals, effecting foreign gene expression in liver, blood vessels, and mammary tissues. Recombinant adenovirus and herpes simplex virus vectors have been utilized effectively for *in vivo* gene transfer into lung and brain tissues, respectively. [Applicants' emphasis]

The Yang reference cites numerous reports of gene transfer and regulation of foreign gene expression in various different tissues. Clearly, vectors useful for gene therapy were available to those skilled in the art by 1995.

The Examiner also argues that specification does not enable treatment of bone loss using the carboxy terminal deleted OPG polypeptides. Applicants have clearly shown in Table 1 on p. 123 that these OPG polypeptides are biologically active yet the Examiner alleges that this "may not be sufficient". If experimental verification of activity of OPG polypeptides within the scope of the claims is not sufficient, it is unclear what showing by Applicants would be needed to support enablement of the claimed polypeptides. It is maintained that the

application teaches the claimed OPG polypeptides in sufficient detail to allow one to practice the full scope of the invention.

Applicants maintain that the Examiner's reasoning lacks support in fact and in law and that a *prima facie* case of nonenablement has not been established. In view of the above remarks, Applicants respectfully request that the rejection be withdrawn.

If it should be determined that a *prima facie* case of nonenablement has been established by the Examiner, Applicants submit herewith as Exhibit B a Declaration by Dr. Jackie Z. Sheng. The Declaration states that introduction of an adenovirus vector expressing a human OPG fusion protein protects against bone loss in ovariectomized rats. These results are also shown in the publication by Bolon et al., (Molecular Therapy 3, 197-205 (2001)) attached hereto as Exhibit C. It is believed that the results presented in the Declaration and the publication are sufficient to rebut any *prima facie* case of nonenablement should such a case be established.

Claims 61-68 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite. Applicants have amended Claims 61 and 62 in order to clarify and more distinctly claim the invention. It is requested that the rejection be withdrawn.

CONCLUSION

Claims 61-68 are in condition for allowance and an early notice thereof is solicited.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Figure 1. A. (SEQ ID NOS: 169 & 170) FASTA analysis of novel EST LORF. Shown is the deduced FRI-1 amino acid sequence aligned to the human TNFR-2 sequence. B. (SEQ ID NOS: 119 & 171) Profile analysis of the novel EST LORF shown is the deduced FRI-1 amino acid sequence aligned to the TNFR-profile. C. Structural view of TNFR superfamily indicating region which is homologous to the novel FRI-1.

Figure 2. Structure and sequence of full length rat OPG gene, a novel member of the TNFR superfamily. A. Map of pMOB-B1.1 insert. Box indicates position of LORF within the cDNA sequence (bold line). Black box indicates signal peptide, and gray ellipses indicate position of cysteine-rich repeat sequences. B, C. (SEQ ID NOS: 120 & 121) Nucleic acid and protein sequence of the Rat OPG cDNA. The predicted signal peptide is underlined, and potential sites of N-linked glycosylation are indicated in bold, underlined letters. D, E. (SEQ ID NOS: 128, 129, 130, 131, 132, 133, 234, 135 & 136) Pileup sequence comparison (Wisconsin GCG Package, Version 8.1) of OPG with other members of the TNFR superfamily, fas (SEQ ID NO:128); tnfr1 (SEQ ID NO: 129); sfu-t2 (SEQ ID NO:130); tnfr2 (SEQ ID NO:131); cd40 (SEQ ID NO:132); osteo (SEQ ID NO:133); ngfr (SEQ ID NO:134); ox40 (SEQ ID NO:135); 41bb (SEQ ID NO:136).

Figure 9. Structure and sequence of mouse and human OPG cDNA clones. A, B. (SEQ ID NOS: 122 & 123) Mouse cDNA and protein sequence. C, D. (SEQ ID NOS: 124 & 125) Human cDNA and protein sequence. The predicted signal peptides are underlined, and potential sites of N-linked glycosylation are indicated in bold. E, F. (SEQ ID NOS: 121, 123 & 125) Sequence alignment and comparison of rat, mouse and human OPG amino acid sequences.

Figure 10. (SEQ ID NOS: 126 & 172) Comparison of conserved sequences in extracellular domain of TNFR-1 and human OPG. PrettyPlot (Wisconsin GCG Package, Version 8.1) of the TNFR1 and OPG alignment described in example 6. Top line, human TNFR1 sequences encoding domains 1-4. Bottom line, human OPG sequences encoding domains 1-4. Conserved residues are highlighted by rectangular boxes.

Figure 12. (SEQ ID NOS: 138 & 139) Structure of OPG cysteine-rich domains. Alignment of the human (top line [SEQ ID NO:136]) and mouse (bottom line) OPG amino acid sequences highlighting the predicted domain structure of OPG. The polypeptide is divided into two halves; the N-terminus (A), and C-terminus (B). The N-terminal half is predicted to contain four cysteine rich domains (labeled 1-4). The predicted intrachain disulfide bonds are indicated by bold lines, labeled "SS1", "SS2", or "SS3". Tyrosine 28 and histidine 75 (underlined) are predicted to form an ionic interaction. Those amino acids predicted to interact with an OPG ligand are indicated by

bold dots above the appropriate residue. The cysteine residues located in the C-terminal half of OPG are indicated by rectangular boxes.

Figure 14. Expression of human OPG in E. coli. A. (SEQ ID NOS: 127 & 137) Construction of a bacterial expression vector. The LORF of the human OPG gene was amplified by PCR, then joined to a oligonucleotide linker fragment (top strand is SEQ ID NO:137; bottom strand is SEQ ID NO:127), and ligated into pAMG21 vector DNA. The resulting vector is capable of expressing OPG residues 32-401 linked to a N-terminal methionine residue. B SDS-PAGE analysis of uninduced and induced bacterial harboring the pAMG21-human OPG -32-401 plasmid. Lane 1, MW standards; lane 2, uninduced bacteria; lane 3, 30°C induction; lane 4, 37°C induction; lane 5, whole cell lysate from 37°C induction; lane 6, soluble fraction of whole cell lysate; lane 7, insoluble fraction of whole cell lysate; lane 8, purified inclusion bodies obtained from whole cell lysate.

Figure 16. Pulse-chase analysis of recombinant murine OPG produced in CHO cells. CHO cells were pulse-labeled with ^{35}S-methionine/cysteine, then chased for the indicated time. Metabolically labeled cultures were separated into both conditioned media and cells, and detergent extracts were prepared from each, clarified, then immunoprecipitated with anti-OPG antibodies. The immunoprecipitates were the resolved by SDS-PAGE, and exposed to film. Top left and right panels (A); samples analyzed under non-reducing conditions. Lower left and right panels (B); samples analyzed under reducing conditions. Top and bottom left panels; Cell extracts. Top and bottom right panels; Conditioned media extracts. The relative mobility of the 55 kd monomeric and 100 kd dimeric forms of OPG are indicated by arrowheads.

Figure 23. Effects of OPG on calvarial osteoclasts in control and IL1-treated mice. Histological methods for analyzing mice calvarial bone samples are described in Example 11B. Arrows indicate osteoclasts present in day 2-treated mice. Calvarial samples of mice receiving four PBS injections daily (A), one injection of IL-1 and three injections of PBS daily (B), one injection of PBS and three injections of OPG daily (C), one injection of IL-1 and three injections of OPG daily (D).

61. (amended) A method for treating bone loss in a mammal comprising administering to the mammal an expression vector comprising a nucleic acid encoding osteoprotegerin and expressing osteoprotegerin having the activity of inhibiting bone resorption.

62. (amended) The method of Claim 61 wherein the nucleic acid sequence is selected from the group consisting of:

a) a nucleic acid encoding a polypeptide comprising the amino acid sequence from residues 1 to 401 or from resides 22 to 401 as shown in Figure 9C-9D (SEQ ID NO:124);

b) a nucleic acid encoding [the polypeptide as in (a) wherein the] a polypeptide [comprises from] comprising a deletion of 1 to 216 amino acids residues [deleted] from the carboxy terminus of the polypeptide as in (a); and

c) a nucleic acid which hybridizes under high stringency conditions of 5XSSC, 50% formamide and 42°C with the nucleic acid set forth in (a) and (b) wherein the hybridizing nucleic acid encodes a polypeptide having the activity of inhibiting bone resorption.